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DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland

ON THE PRESERVATION OF THE ANTIGEN (T. NICHOLS)  
BY THE IMMUNOFLUORESCENT TREPONEMAL  
ANTIBODY TEST (FTA)

Giornale de igiene e medicina  
preventiva (Genova)

(Journal of hygiene and pre-  
ventive medicine (Genoa)

6: 180-85, 1965

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Among the treponemal tests for the serological diagnosis of lues, the immunofluorescence (Fluorescent Treponemal Antibody or FTA) test is frequently used by now. Based on the indirect method of Coons (reference 17), it has been applied to the study of luetic antitreponemal antibodies by Deacon, Falcone, and Harris (reference 6).

The original technique has successively undergone several modifications, such as using the serums in dilutions of 1:200 (FTA 200), instead of the initial 1:5 dilution in order to reduce non-specific reactions (reference 7); a longer time of contact between antigen and serum under study (reference 11); the method of adhesion of the antigen on slides and the best system of reading them (reference 8).

The diagnostic value of the FTA test in the various stages of luetic infection in man and in experimental work is amply documented by now (references 1, 2, 3, 4, 5, 9, 12, 13, 14, 15, 16).

From the studies mentioned above, the test is also indicated for primary lues because of the rapid appearance of

the fluorescent antibodies compared with the flocculant antibodies and deviating antilipoidic and antiproteic complement, and particularly in regard to the immobilization.

It must be remembered that the FTA test does not require excessive equipment for its execution, and thus it is possible to be done in a great number of laboratories compared with the Nelson and Mayer immobilization test, regarding which it is also more economical and safer because of the use of dead treponemas although it is more delicate to execute. But many factors may negatively influence the success of the reaction, some of which are inherent in the technique, others in the reagents used, especially human antiglobulin serum with fluorescein isothiocyanate. For these reasons, standardization of the method and of the materials has already been considered (reference 2).

Another element of fundamental importance is represented by treponemal suspensions to be used as an antigen, in relation to methods used to preserve it.

On this point, there is no consensus among the various authors. For example, Nelson and his colleagues consider the use of lyophilized antigens satisfactory (reference 13), while Niel and his colleagues advise against using lyophilized or frozen antigens and propose using treponemal suspensions in physiological solution, preserved at + 4°C without adding merthiolate which they consider responsible for the abnormal reaction (reference 12).

We therefore thought of conducting some comparative tests using antigens preserved in different ways in order to establish which of the many methods should be the preferred method.

The tests conducted and the results obtained are the subject of this note.

## PERSONAL EXPERIENCE

Antigens: in carrying out our tests, antigens of the following characteristics were used:

- 1) Treponemal suspension lyophilized commercially;
- 2) Treponemal suspension in saline solution buffered with phosphate of pH 7.2, merthiolate 1:5,000, and preserved at + 4°C;
- 3) Treponemal suspension according to Nelson and Mayer with streptomycin 1:1,000, and preserved at + 4°C;
- 4) Treponemal suspension as in number 3, but set on the special slides and preserved at - 20°C.

Antigens numbers 2, 3, and 4 we prepared ourselves by extracting the treponemas from the syphilitic orchitis of rabbits and adjusting their microbe content to 40-50 treponema per microscopic field.

Non-human globulin conjugated with fluorescein isothiocyanate: a lyophilized commercial product was used.

Pools of reactive and non-reactive human serums: these pools had been previously titrated by the FTA 200 in comparison with fresh treponemas and were successively subdivided into small fractions and preserved at - 20°C.

With the antigen preparations mentioned above, immunofluorescence tests were periodically conducted with reactions of the same reactive and non-reactive pools. The technique used is "Laboratory Procedures of Modern Syphilis Serology" (reference 10) for FTA 200, with some modifications on the fixation or adhesion of the antigen on the slides, the best system of reading (reference 8), and showing negative results with the sign -- and positive results with 1-4 signs of + according to the intensity of the fluorescence.

Results obtained are reported in Table No. 1.

Table No. 1. Results obtained in FTA 200 reactions using antigens preserved in different ways.

Controlli dopo: (a)	(f) 15 giorni Pool sieri		30 giorni Pool sieri		60 giorni Pool sieri		90 giorni Pool sieri		120 giorni Pool sieri		180 giorni Pool sieri	
	R	NR	R	NR	R	NR	R	NR	R	NR	R	NR
(b) Antigene liofilizzato	+++	-	+++	-	+++	-	+++	-	+++	-	+++	-
(c) Antigene in soluzione salina tamponata pH 7.2 + merthiolato e conservato a + 4 °C.	+++	-	+++	-	+++	-	+++	-	+++	-	++	-
(d) Antigene in terreno di Nelson-Mayer + streptomycina e conservato a + 4 °C.	+++	-	+++	-	+++	-	+++	-	+++	-	++	-
(e) Antigene in terreno di Nelson-Mayer, fissato su vetrino e conservato a - 20 °C.	+++	-	++	-	+++	-	+++	-	++	-	++	-

Key: (a) Controlled after: (b) Lyophilized antigen  
 (c) Antigen in saline solution, buffered, pH 7.2 + merthiolate, and preserved at + 4°C (d) Antigen according to Nelson and Mayer + streptomycin, and preserved at + 4°C. (e) Antigen according to Nelson and Mayer, adhered to slide and preserved at - 20°C  
 (f) 15 days pool serums

From an examination of the data reported in the Table comes the consideration that the lyophilized antigen, presenting only weak reactivity compared with that in liquid suspension, preserves it for a longer time.

A second consideration is that among the antigens preserved at + 4°C, the best results were obtained with the

treponemal suspension in saline solution, pH 7.2 + merthiolate, which maintained a high reactivity about three months, while those without merthiolate according to Nelson and Mayer's method demonstrated a shorter time (60 days).

The third consideration possible is to prepare slides with antigens and preserved at - 20°C for only a short period of time (about 15 days).

In conclusion, it is considered that preference should be accorded to treponemal suspensions in a liquid medium which can be used in a period of about three months, particularly if preserved at + 4°C and with the addition of merthiolate which gave us no inconvenience. In the second place, preference should be given to lyophilized suspensions which preserve their reactivity for a much longer time, even if this reactivity is slightly less than that of the antigens in liquid mediums. On the other hand, treponemal suspensions on slides and preserved at - 20°C are to be rejected since their reactivity is maintained for too short a time.

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